

Casting SDS-PAGE gel

Introduction

How to make SDS-PAGE gels

Materials

- > 10% APS
- > TEMED
- > 40% acrylamide
- > 4x separating buffer (1,5 M Tris-HCl, pH 8,8)
- > 2x stacking buffer (0,25 M Tris-HCl, pH 6,8)
- > sterile water

Procedure

Casting the gel

1. Check that the glasses are clean. Wash and rinse with water and ethanol if necessary.
2. Set up the casting gear. Press glasses against each other so that the lower end is even (check by placing on the table for example). It is best to set up the glasses in the green holder and then levelling them on the table. You may check with water that the set-up doesn't leak. Remove the water by pouring and Whatman filter paper.
3. Mark the border of separating and stacking gel by measuring 0.5 cm below the comb. This is the lower end of green thing.
4. Mix the separating gel according to the table below. The mixture is enough for two gels. APS and TEMED are responsible for the polymerization so add these last.

Table1						
	A	B	C	D	E	F
1	acrylamide end concentration %	4x separating buffer	H2O (ml)	40% Acrylamide (ml)	10% APS (ul)	TEMED (ul)
2	12,5	2,5	4,375	3,125	60	12,5

5. Pour or pipette the mixture between the glasses till the marking.
6. Add 500 µl saturated butanol or isopropanol evenly on top of the gel.
7. Let the separating gel solidify (takes around 45 minutes).
8. Mix the stacking gel according to the scheme. The amounts below are for four gels.

Table2						
	A	B	C	D	E	F
1	acrylamide end concentration %	2x stacking buffer	H ₂ O (ml)	40% Acrylamide (ml)	10% APS (ul)	TEMED (ul)
2	3	3,8	3,2	0,6	60	12,5

9. Pour butanol/isopropanol out and rinse with ethanol/water. Dry with Whatman-pieces.
10. Add the stacking gel –mixture on top of the gels. Push the combs inside and let the stacking gel solidify (for about 20 minutes).
11. You can use the gel for running rigght away or store it at +4°C wrapped in moist paper and plastic/aluminium foil